ASSESSMENT OF FUNGAL PATHOGENS AS BIOCONTROL AGENTS OF *MYRIOPHYLLUM SPICATUM*

by

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Abstract

Nine fungal isolates, which had shown potential to control sections of *Myriophyllum spicatum,* were screened against whole actively growing plants. None of the isolates produced any control capabilities . Though there could be a use for the pathogens in conjunction with other methods of control, there use alone is unlikely to be sufficient.

Keywords

Myriophyllum spicatum, Biocontrol agents, fungal pathogens

Introduction

Myriophyllum spicatum L. (or Eurasian watermilfoil) is a member of the Haloragidaceae family. It is a submerged aquatic plant which grows in a wide range of environmental conditions, in both fresh and brackish waters. In weedy situations, it is very fast growing, forming dense mats of foliage that interfere with the normal usage of water courses. Reproduction is by fragmentation of stems and the development of over-wintering buds; seed formation also occurs but may play little part in the spread of the weed.

M. spicatum is widely distributed throughout the U.K., with records from Cornwall through to the Outer Hebrides: and occurs in most European countries from Scandinavia in the North to Sicily in the South (Kew Herbarium Records). It also occurs in most of Asia as well as in East Africa (Harley & Forno, 1990). Although locally common throughout the natural range; it is rarely dominant and has never been reported as a weed problem.

M. spicatum has been a problem in the United States since the 1930's (Harley and Forno, 1990). In the 1950's and 1960's it became a serious ecological and economical weed in larger bodies of water in North America. As an ecological problem, *M. spicatum* can greatly reduce the numbers of naturally occurring aquatic plant species, with records of a fall in species number from 20 to 9 in a two year period, with *M. spicatum* coverage increasing from 2% to 20-45% over the same period (Madsen, Sutherland, Bloomfield, Eichler & Boylen, 1990).

Attempts to control *M. spicatum* have involved both mechanical and chemical methods. Mechanical clearance can be cheaper than chemical alternatives, but needs to be carried out at least twice during the summer to produce a reasonable reduction in plant biomass. Herbicide applications have been successful, both underwater applications made by boat and aerial applications can give good control. However, because of environmental concerns applications of chemical herbicides need careful consideration. Due to the dilution from a body of water, large amounts of herbicide need to be applied, and, if control is not sufficient, reinfestation can be rapid. In addition, the chemical has to be specific, and be persistent enough to control the weed with no residual activity.

Many of the early investigations into biological control agents for *M. spicatum* concentrated on insects. Species on other *Myriophyllum* spp. from within the USA have been identified as possible control agents. A pyralid moth, *Acentria nivea,* found in stands of *Myriophyllum exalbescens* in the St Lawrence River caused leaf loss and girdling of stems (Batra, 1977). Surveys predominantly for insect agents have also been carried out in Pakistan, Bangladesh and much of Eastern Europe and Asia (Final report, CIBC Pakistan station 1965-1970; Harley &Forno, 1990). However, many of the insects found proved to be non-specific to the target weed and hence of limited use as biological control agents.

Use of pathogens has long been regarded as a good potential method of biological control for *M. spicatum* (Freeman &Charudattan, 1980). Work has been undertaken on isolating and assessing fungal pathogens from within the USA: *Fusarium sporotrichoides* was tested at Wisconsin, but, though capable of causing lesions, failed to control the weed in large scale tests (Andrews &Hecht, 1981; Charudattan, 1990). *Acremonium*

curvulum was also found in association with *M. spicatum* growing both epiphytically and endophytically. The presence of the fungus appeared to cause an increase in the plants decline when it was environmentally or mechanically stressed, but in Laboratory tests could not control the plant (Andrews, Hecht &Bashirian, 1982).

A fungal pathogen, *Colletotrichum gloeosporioides,* found on *M. spicatum* in Wisconsin, was evaluated for it' potential to control *M. spicatum.* However, except in senescing tissue the fungus was confined to the plant surface and failed to penetrate beyond the epidermis, and was regarded an insufficient to be used to control plants in realistic conditions (Smith, Slade, Andrews and Harris, 1989). *Mycoleptodiscus terrestris,* from the southern States has also been tested against *M. spicatum* and a series of aquatic weeds and terrestrial crop plants, and has been shown to be virulent and reasonably specific (Verma & Charudattan 1993).

M. spicatum constitutes part of the background or natural aquatic flora throughout most of Europe and rarely reaches weed status. However, some of these ecosystems (in Central Western Europe) have recently been invaded by the North American exotic species *Myriophyllum heterophyllum* (Spangehl & Scharrenberg, 1986). Domination by the latter species would indicate that a different spectrum of natural enemies occurs in Europe and that a search for a fungal biological control agent for *M. spicatum* within Europe would be beneficial.

During an initial two-year project, sites for pathogen surveys of *Myriophyllum spicatum* were selected, with the aid of literature searches and distribution data from Kew herbarium, the Institute of Terrestrial Ecology and National Water Boards. Surveys were carried out in the UK and mainland Europe (France, Switzerland, Germany, Luxembourg, The Netherlands, Italy, Slovenia, Austria, Spain and Portugal).

Diseased and healthy tissues were collected from nearly 200 sites, covering a range of habitats and environmental conditions. Isolations were made on selective media and over 400 potential pathogens in 38 genera were obtained in pure culture, and identifications were confirmed, wherever possible at the CAB International Mycological Institute.

Representatives of all genera have been screened against small sections of *M. spicatum* (a total of 358 isolates), using stem sections, in a primary screen. At the completion of this initial phase, several isolates (Table 1) had shown themselves to be pathogenic to small section of *M. spicatum,* and this three month project was to confirm their pathogenicity on larger actively growing plants, an area in which other pathogens have sometimes failed to cause significant damage.

Table 1

isolate number	isolation site	isolate name	symptoms from detached section tests
Mir 134a (IMI 368660)	Oversley Edge River, England	*Cryptosporiopsis* sp.	Death of old tissue and reduction in new growth
Mir 3iii (IMI 359296)	Slapton Ley, England	*Embellisia telluster*	Death of old tissue and reduction in new growth (re isolated, x2)
Mir 3a reisolate		*Embellisia telluster*	
Mir 16 (IMI 374409)	Wicken Fen, England	*Fusarium oxysporum*	Death of old tissue and reduction in new growth
Mir 49a (IMI 374408)	River Hart, England	*Fusarium flocciferum.*	Death of old tissue and reduction in new growth (re isolated, x2)
Mir 51 (IMI 362886)	Basingstoke Canal, England	*Glomerella cingulata*	Reduction in rate of growth (x2)
Mir 59 (IMI 368844)	Dockens Water, England	*Geotrichum candidum*	Death and necrosis of tissue and reduction in growth (re isolated)
Mir 59d reisolate		*Geotrichum candidum*	
Mir 64a (IMI 368840)	Llan Bwch-llyn Lake, Wales	*Coniothyrium fuckelii*	Necrosis of tissue no effect on new growth (re isolated)
Mir 64d reisolate		*Coniothyrium fuckelii*	
Mir 80b (IMI 364456)	Grasmere, England	*Cylindrocarpon destructans*	Necrosis of old tissue and reduction in rate of growth (re isolated, x2)
Mir 80c (IMI 374407)	Grasmere, England	*Torula herbarum**	Death of old tissue and reduction in new growth (re isolated, x2)
Mir 93b (IMI 368670)	Lac de Longemer, France	*Gliocladium roseum*	Death of old tissue, no effect on new growth (re isolated)
Mir 93g reisolate		*Gliocladium roseum*	

* This was a resent identification, but *Torula herbarum* is known as a good sporulater and Mir 80c is not therefore the identification is not certain

Materials and Methods

Sections of plants up to 30cm in length were rooted in gravel in containers of water, three plants per container. These were inoculated with a 10^6 to 10^9 spores per ml suspension (dependent upon sporulation of the isolate) and a 4cm agar plug with mycelium also added. The containers were kept at 25°-20°C with 12hr light, six uninoculated controls were included. After three weeks plants were visually assessed for any indication of infection. After eight weeks plants were assessed and sections plated onto TWA with antibiotics for identification and proof of pathogenicity (Koch's postulates).

In a second experiment plants of approximately 10cm in length were rooted in gravel in containers, three plants per container. Inoculations were as previous, but before inoculation a large proportion of the water was siphoned off, leaving approximately 200mls. Seven days after inoculation, containers were refilled. Six weeks a second spore suspension was added to the containers, at 10^6 spores per ml. After a further eight weeks plants were assessed and sections plated onto TWA with antibiotics for identification and proof of pathogenicity (Koch's postulates).

Not all isolates were used in both tests.

Results and Discussion

In the first test, symptoms after eight weeks showed little effect from any of the pathogens on the plants (Table 2). All roots were healthy and stems did not differ from control plants. In two cases (Mir 51 *Glomerella cingulata* and Mir 64d *Coniothyrium fuckelii*) plants were smaller than the controls, but showed no other symptoms or signs of infection and no re-isolation of the pathogen was possible. In two cases (Mir 80b *Cylindrocarpon destructans* and Mir 93b *Gliocladium roseum*) the inoculated pathogen could be re-isolated from two thirds of the inoculated plants, but the plants showed no sign of infection and no loss of growth. In a further two cases (Mir 59d *Geotrichium candidum* and Mir 64a *Coniothyrium fuckelii*) the inoculated pathogen could be re-isolated from one third of the inoculated plants, with Mir 64a again plants showed no sign of infection and no loss of growth. The plant from which Mir 59d was re-isolated had an increase in the percentage of the stem browning compared to the other inoculated plants.

In the second test, symptoms were not clear even after 14 weeks (Table 3), however, re isolation of the isolates from plant tissue was more common than in the first test, possibly due to the higher concentrations which were used. In four cases (Mir 3a *Embellisia telluster*, Mir 49a *Fusarium flocciferum*, Mir 80c *Torula herbarium* and Mir 80b *Cylindrocarpon destructans*), plants were slightly smaller than the controls and some sign of blackening or damage could be seen on either the stems or the roots. In only two of these (Mir 3a *Embellisia telluster* and Mir 49a *Fusarium flocciferum*) could the isolate be regained. All other plants from which re isolations were made showed no symptoms.

Though most of the isolates appear able to invade the plants tissue, damage caused to whole plants appears slight and inconsistent. It seems unlikely, given the evidence to date that any of the pathogens would be capable of producing significant control of Stands of *M. spicatum*. There ability to control small sections of the plants but not whole plants would tend to indicate that they are not primary pathogens, but endophytic fungi capable of causing damage only if the plant is otherwise stressed.

Table 2: Results of first test

isolate number	isolate name	symptoms	isolation
Mir 134a (IMI 368660)	*Cryptosporiopsis* sp.	roots healthy, base 10% of stems beginning to go brown, growing tips healthy, no visible lesions or necrotic patches, no loss in mass or reduction in growth compared to controls.	No isolation
Mir 3a re isolate	*Embellisia telluster*	roots healthy, base 50% of stems beginning to go brown, growing tips healthy, no visible lesions or necrotic patches, no loss in mass or reduction in growth compared to controls.	No isolation
Mir 3iii (IMI 359296)	*Embellisia telluster*	roots healthy, base 10% of stems beginning to go brown, growing tips healthy, no visible lesions or necrotic patches, no loss in mass or reduction in growth compared to controls.	No isolation
Mir 49a (IMI 374408)	*Fusarium flocciferum*	roots healthy, base 50% of stems beginning to go brown on 60% of replicates remainder all green, growing tips healthy, no visible lesions or necrotic patches, no loss in mass or reduction in growth compared to controls.	No isolation
Mir 51 (IMI 362886)	*Glomerella cingulata*	roots healthy, base 10% of stems beginning to go brown, growing tips healthy, no visible lesions or necrotic patches, plants smaller than most ,but flowering	No isolation
Mir 59 (IMI 368844)	*Geotrichum candidum*	roots healthy, base 50% of stems beginning to go brown, one growing tip dead remainder healthy, no visible lesions or necrotic patches, no loss in mass or reduction in growth compared to controls.	No isolation
Mir 59d re isolate	*Geotrichum candidum*	roots healthy, base 2-50% of stems beginning to go brown, new shoots appearing at base of plants and heavy rooting from brown stems, growing tips healthy, no visible lesions or necrotic patches, no loss in mass or reduction in growth compared to controls, one plant flowering	Re-isolation from 1/3 plants
Mir 64a (IMI 368840)	*Coniothyrium fuckelii*	roots healthy, base 10-20% of stems beginning to go brown, growing tips healthy, no visible lesions or necrotic patches, no loss in mass or reduction in growth compared to controls.	Re-isolation from 1/3 plants
Mir 64d re isolate	*Coniothyrium fuckelii*	roots small, base 50% of stems beginning to go brown, growing tips healthy, no visible lesions or necrotic patches, plants small.	No isolation
Mir 80b (IMI 364456)	*Cylindrocarpon destructans*	roots healthy, base 40% of stems beginning to go brown, growing tips healthy, no visible lesions or necrotic patches, no loss in mass or reduction in growth compared to controls, one plant flowered.	Re-isolation from 2/3 plants

isolate number	isolate name	symptoms	isolation
Mir 80c (IMI 368033)	*Torula herbarum*	roots healthy, base 20% of stems beginning to go brown, growing tips healthy, no visible lesions or necrotic patches, no loss in mass or reduction in growth compared to controls.	No isolation
Mir 93b (IMI 368670)	*Gliocladium roseum*	roots healthy, re-rooting from stems, base 30% of stems beginning to go brown, growing tips healthy, no visible lesions or necrotic patches, no loss in mass or reduction in growth compared to controls, plants flowering.	Re-isolation from 2/3 plants

Table 3: Results Second Test

isolate number	isolate name	symptoms	isolation
Mir 3iii (IMI 359296)	*Embellisia telluster*	1 plant with small root system, remainder roots healthy. All stems healthy, no browning.	
Mir 3a re isolate	*Embellisia telluster*	1 plant with small root system, remainder roots healthy. All stems healthy, no browning.	Re isolated from 1/3 plants
Mir 16 (IMI 374409)	*Fusarium oxysporum*	Roots healthy. Stems healthy. No sign of any damage to plants.	Re isolated from 3/3 plants
Mir 49a (IMI 374408)	*Fusarium flocciferum*	1 plant with small root system and damaged stem. Remaining plants both root and stem healthy.	Re isolated from 1/3 plants
Mir 51 (IMI 362886)	*Glomerella cingulata*	Roots healthy. 1 stem small but healthy, remaining stems normal and healthy.	
Mir 59 (IMI 368844)	*Geotrichum candidum*	Roots healthy. Stems healthy. No sign of any damage to plants.	Re isolated from 1/3 plants
Mir 64d re isolate	*Coniothyrium fuckelii*	Roots healthy. Stems healthy. No sign of any damage to plants.	Re isolated from 3/3 plants
Mir 80c (IMI 368033)	*Torula herbarium*	1 plant with small dark roots and small stems. Remaining plants are healthy.	
Mir 80b (IMI 364456)	*Cylindrocarpon destructans*	1 plant with small roots and small blackened stems. Remaining plants are healthy.	
Mir 93b (IMI 368670)	*Gliocladium roseum*	Roots healthy. Stems healthy. No sign of any damage to plants.	Re isolation from 2/3 plants
Mir 93g re isolate	*Gliocladium roseum*	Roots healthy. Stems healthy. No sign of any damage to plants.	Re isolation from 2/3 plants
Mir 134a (IMI 368660)	*Cryptosporiopsis* sp.	Roots healthy. Stems healthy. No sign of any damage to plants.	

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